Response of a Pearly Eye Melon Fly
*Bactrocera cucurbitae* (Coquillett) (Diptera: Tephritidae)
Mutant to Host-Associated Visual Cues

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Abstract. We report on a pearly eye mutant (*PEM*) line generated from a single male of *Bactrocera cucurbitae* collected in Kapoho, Hawaii. Crossing experiments with colony wild-type flies indicate that the locus controlling this trait is autosomal and the mutant allele is recessive. Experiments with females to assess response to visual oviposition cues (shape and color) suggest that *PEM* flies are at least unresponsive to color, and likely also unable to perceive visual shape cues. This phenotype has been described from field collection before, but its visual abilities have not previously been tested. The rediscovery of the *PEM* phenotype and results of the vision test support the hypothesis that the *PEM* trait has significant negative fitness consequences in the field, and that the recessive allele resulting in this phenotype probably occurs at a low frequency in nature.

Key words: color, shape, mark-release-recapture, genetic marker, SIT

*Bactrocera cucurbitae* (Coquillett) (melon fly) is an important pest species in Hawaii, introduced in 1895 (Back and Pemberton 1918). It is considered oligophagous, breeding predominately on cucurbits, and has considerable invasive potential (Duyck et al. 2004, Dhillon et al. 2005). Despite its relatively long life and generally wide host range, eradication of this species from the Okinawa Islands was effective, mainly via an integrated pest management approach that included utilization of the Sterile Insect Technique (SIT) (Koyama et al. 2004).

Eye mutants of *B. cucurbitae* have been found or created over the years. Naturally occurring “yellow-eyed” melon flies were described by Kobayashi et al. (1973). A “light eye” (*le*) mutant was later produced by exposing wild-type laboratory lines to ethyl methanesulfonate (Saul and McCombs 1992), and a “white eye” (*we*) mutant was reported from exposure to a cobalt-60 source, as well as a spontaneous “yellow eye-2” (*ye*-2) mutant (McCombs et al. 1996, Peabody et al. 2009). Harris et al. (2006) reported on the field discovery of a “Pearly eye” mutant (*PEM*) from ivy gourd (*Coccinia grandis* (L.)) collected in Laie, Oahu island. Both *PEM* and *ye*-2 were determined to be autosomal recessive traits, and both were kept as true-breeding strains in the laboratory for some years.

Genetic markers such as light eyes in melon flies have been suggested to be potentially useful as markers for flies released under an SIT program or for field research, as well as to create genetic sex-sorting systems to increase the efficiency of SIT (McCombs et al. 1996, Harris et al. 2006). Less frequently or carefully as-
essed is the fitness impact of the genetic traits in question, though the need to assess relative fitness has been mentioned (Harris et al. 2006).

In this study we report on the probable rediscovery of PEM from field collections conducted in June 2014 in Kapoho on Hawaii island. We found a single male PEM in papaya from that collection. Following Harris et al. (2006), we conducted an experiment to assess the genetic basis of the PEM trait for comparison with the original report. All B. cucurbitae breeding and rearing was conducted using standard methodology (Vargas 1989). We conducted reciprocal outcrosses starting from a single parental pair of PEM and wild-type colony (NE) reared B. cucurbitae from the research colony at the Daniel K. Inouye US Pacific Basin Agricultural Research Center (DKI-PBARC) in Hilo, Hawaii. Crosses were conducted with single pairs in cubical cages (25 cm x 25 cm x 25 cm). For each set of offspring we allowed full siblings to breed to produce an F2 generation, where we observed a 3:1 phenotypic ratio of NE:PEM in both reciprocal crosses (Table 1). This outcome shows that the PEM locus is not sex linked, and that the allele encoding PEM is recessive. These results are in agreement with the findings of Harris et al. (2006), suggesting that the current line is a probable rediscovery of the PEM trait they described.

In addition to the crossing experiments, we tested visual responses in female PEM B. cucurbitae compared with wild type B. cucurbitae from the DKI-PBARC colony using fruit-mimic spheres to understand the fitness consequences of the PEM trait (Piñero et al. 2006). One hundred females of each type were placed in separate 1m³ cages under a simple roof outdoors at DKI-PBARC between 21 December 2015 and 28 January 2016. Cages were placed 2 m apart to ensure similar lighting conditions. For each of seven experimental dates (replicates) one yellow and one black Tanglefoot-coated (Contech Enterprises Inc, Victoria BC) fruit mimic spheres (8 cm diameter) were hung from a top-mounted carousel, which was rotated at 2 RPM inside each of the two cages.

Females were sorted on the day of the experiment at 4°C. 100 females of each type were then placed in separate 20-cm powdered flight tubes (Boller et al. 1981), which were introduced to separate cages around 08:30 h. Females were allowed to fly from the flight tubes and potentially become caught on the Tanglefoot-coated spheres for six hours. After this period, the number of non-fliers left in each flight tube was recorded, as were the number of females trapped on each fruit-mimic sphere.

Flight ability was similar between the PEM and regular colony flies (Table 2). However, we observed a much lower proportion of the PEM females on either of the spheres (on average 6 of 94 flying individuals) compared with NE flies (average 50 of 97 flying females). Comparing the spheres, we found an average three times as many NE B. cucurbitae on yellow spheres compared with black, a significant difference (one-tailed paired t test, $t = 5.38$, d.f. = 6, $p < 0.002$). For the PEM females there was no statistically significant difference in the low number of flies caught in the yellow versus black spheres (one-tailed paired $t$ test, $t = 1.216$, d.f. = 6, $p = 0.270$). Full details are in Table 2.

We conclude from the visual test that the PEM line is unable to see colors (due to equivalent catches on spheres of each color), and probably unable to respond to visual shape cues also (overall low number on the spheres). Light detection through ocelli likely occurs in PEM flies, as they successfully left the flight tube at about the same rate as the wild-type B. cucurbitae. However, it is clear that they did not respond to visual cue stimulation
Table 1. Reciprocal single-pair crosses of Pearly Eyed Mutant (PEM) and wild-type colony (NE) *B. cucurbitae*. $X^2$ goodness of fit tests to a 3:1 *NE:PEM* ratio in the F2 generation of the heterotypic crosses confirm that the mutant allele is recessive (i.e. observed ratio does not differ from 3:1 expectation): PEM (m) x NE (f) F2 $X^2 = 0.207$, d.f. = 1, $p = 0.65$; NE (m) x PEM (f) F2 $X^2 = 0.01$, d.f. = 1, $p = 0.75$.

<table>
<thead>
<tr>
<th>Cohort</th>
<th>Generation</th>
<th>Phenotype cross (m x f)</th>
<th>No. pupae</th>
<th>NE-m</th>
<th>NE-f</th>
<th>PEM-m</th>
<th>PEM-f</th>
<th>Phenotype ratio (NE:PEM)</th>
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<tr>
<td>1</td>
<td>P</td>
<td>PEM x PEM</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>1</td>
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<td></td>
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<td>2</td>
<td>P</td>
<td>PEM x NE</td>
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<td>1</td>
<td>0</td>
<td>0</td>
<td>1</td>
<td>-</td>
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<tr>
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<tr>
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<td></td>
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<td>9</td>
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<tr>
<td>3</td>
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<td>NE x PEM</td>
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<td>0</td>
<td>1</td>
<td>1</td>
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<td>-</td>
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<td>45</td>
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Table 2. Flight test (FT) results and number of female *B. cucurbitae* caught on yellow and black fruit-mimic spheres after six hours. The overall number of pearly eyed mutants (PEM) leaving tubes was similar to wild type (NE), but many fewer were caught on spheres, and there was not an increased number on yellow versus black spheres as observed with NE females. Response is calculated as (proportion of fliers captured on spheres)*(proportion of sphere capture on yellow).

<table>
<thead>
<tr>
<th>Replicate</th>
<th>NE FT</th>
<th>PEM FT</th>
<th>NE Yellow</th>
<th>NE Black</th>
<th>NE Response</th>
<th>PEM Yellow</th>
<th>PEM Black</th>
<th>Response</th>
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<tbody>
<tr>
<td>1</td>
<td>91</td>
<td>86</td>
<td>47</td>
<td>26</td>
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<td>90</td>
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<td>1</td>
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<td>94</td>
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<td>11</td>
<td>0.31</td>
<td>0</td>
<td>4</td>
<td>0.00</td>
</tr>
<tr>
<td>7</td>
<td>99</td>
<td>97</td>
<td>23</td>
<td>17</td>
<td>0.23</td>
<td>2</td>
<td>0</td>
<td>0.02</td>
</tr>
<tr>
<td>Mean (SD)</td>
<td>97 (4)</td>
<td>94 (5)</td>
<td>38 (10)</td>
<td>12 (12)</td>
<td>0.39 (0.11)</td>
<td>2 (2)</td>
<td>4 (4)</td>
<td>0.03 (0.02)</td>
</tr>
</tbody>
</table>
B. cucurbitae mutant vision

like the wild type colony flies did. We an-
ecdotally observed PEM flies aggregating
at the bottom of the cage, though no flight
ability difference was detected from the
flight tube assays.

Since visual stimuli are thought to be
particularly important in this species,
there is likely to be a very large fitness
cost to the PEM trait in the field (Prokopy
and Owens 1983, Piñero et al. 2006). The
fact that the trait has been found at least
twice under natural conditions suggests
that the recessive allele persists in the wild
population at low frequency, preserved
in heterozygotes (hybrid protection). In
any case, our results suggest PEM would
not be a suitable marker for SIT or field
research, because these B. cucurbitae
would have a difficult time finding host
fruit or mates.

The PEM line described here might be
genetically distinct from the one described
by Harris et al. (2006), though our results
suggest that it is not. Despite being found
on a different Hawaiian Island, the line in
this study was also found to be an auto-
somal recessive trait, and shares the phe-
notype of the original PEM. Additionally,
the current line also changes from pearly
to tan after death like the original, again
suggesting that they are produced by the
same locus. This is in contrast to ye, which
remained unchanged after death, and le,
which was reported to darken in dead flies.
Certainty that the previous and current
PEM lines are the same is not possible
without direct sequencing of both mutants.

Acknowledgments
We thank Jaime Piñero for suggestions
on visual testing and the DKI-PBARC
rearing crew (Mike McKenney and Keith
Shigetani) for providing colony flies for
the experiments. Scott Geib and Sheina
Sim provided helpful comments on an
early draft, and three anonymous review-
ers for further suggestions. This work
was supported by the US Department
of Agriculture, Agricultural Research
Service. Opinions, findings, conclusions
or recommendations expressed in this
publication are those of the authors and
do not necessarily reflect the views of the
USDA. USDA is an equal opportunity
provider and employer.

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